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Award Number: W81XWH-05-1-0204

TITLE: Identification, Characterization and Clinical Development of the New Generation of Breast Cancer Susceptibility Alleles

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REPORT DATE: March 2007

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE (DD-MM-YYYY) 01-03-2007	2. REPORT TYPE Annual	3. DATES COVERED (From - To) 1 Mar 2006 – 28 Feb 2007		
4. TITLE AND SUBTITLE Identification, Characterization and Clinical Development of the New Generation of Breast Cancer Susceptibility Alleles		5a. CONTRACT NUMBER		
		5b. GRANT NUMBER W81XWH-05-1-0204		
		5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) Nazneen Rahman M.D. Ph.D. E-Mail: nazneen.rahman@icr.ac.uk		5d. PROJECT NUMBER		
		5e. TASK NUMBER		
		5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Institute of Cancer Research Sutton, Surrey, London SW7 3RP		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSOR/MONITOR'S ACRONYM(S)		
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				
13. SUPPLEMENTARY NOTES				
14. ABSTRACT Breast cancer is a common disease in women but the causes are still largely unknown. There is considerable evidence to suggest that genetic factors play an important role in causing breast cancer, but the genes involved in the majority of breast cancers are currently unknown. Our aim is to identify genetic factors that increase the chance of breast cancer occurring. We have collected clinical information and samples from over 1500 breast cancer families. We will compare the frequency of genetic factors in these cases with control women without breast cancer. Within the last year we have used this new strategy to identify three new breast cancer predisposition genes, ATM, BRIP1 and PALB2, that each confer a 2-3 fold risk of breast cancer and account for ~3% of excess risk of breast cancer. We have also performed an experiment to evaluate 15,000 coding genetic variants in 864 breast cancer cases and 1498 controls and we are following up these results in additional cases.				
15. SUBJECT TERMS Breast cancer; genetic predisposition; cancer gene.				
16. SECURITY CLASSIFICATION OF: a. REPORT U		17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 30	19a. NAME OF RESPONSIBLE PERSON USAMRMC
b. ABSTRACT U				19b. TELEPHONE NUMBER (include area code)
c. THIS PAGE U				

Table of Contents

Introduction.....	4
Body.....	6
Key Research Accomplishments.....	11
Reportable Outcomes.....	11
Conclusions.....	11
References.....	12
Appendices.....	14
Supporting Data.....	30

INTRODUCTION

Breast cancer is a common disease in women but the causes are still largely unknown. There is considerable evidence to suggest that genetic factors play an important role in causing breast cancer. In the last decade considerable progress has been made and two major breast cancer genes, *BRCA1* and *BRCA2*, have been identified (Rahman and Stratton, 1998). These genes carry a high risk of breast cancer but only account for a very small proportion of breast cancer families. Weaker genes are likely to be involved in the majority of familial breast cancers and some breast cancer cases without a family history of the disease, but few have been identified (Antoniou and Easton, 2003; Meijers-Heijboer et al. 2002).

Our aim is to identify and characterize the genetic factors that increase the chance of breast cancer occurring. We have collected clinical information and samples from over 1500 breast cancer families. We first characterized these for the known breast cancer genes, *BRCA1* and *BRCA2*, with particular emphasis on clarifying the contribution and nature of large rearrangements of these genes, which have recently been identified in some familial breast cancer pedigrees and which are not identifiable by gene sequencing. We will then proceed to try to identify new genes, by comparing the frequency of genetic factors in these cases with control women without breast cancer. Initially we are analyzing genes that we suspect may have a role in breast cancer, because they are related to known breast cancer genes. However over the course of the study we plan to use new technologies to analyze every gene. If we find any variants that are more frequent in breast cancer cases than controls, it suggests that they may be involved in causing breast cancer. We will evaluate these variants in further cases and controls to prove an association with breast cancer and to define the risk and outcomes of carrying the genetic variant(s).

In the last year we have had considerable success in using this strategy for candidate genes to identify two new breast cancer susceptibility genes (*BRIP1* and *PALB2*) and to provide molecular confirmation of the association of *ATM* and breast cancer susceptibility, which has been controversial for nearly two decades (Renwick et al. 2006; Seal et al. 2006; Ahmed and Rahman, 2006; Rahman et al. 2007, see attached papers). We have also made progress in genome-wide surveys analyzing ~15000 single nucleotide polymorphisms in 1864 cases and 1498 controls.

BODY

As part of the program of work we defined five tasks. The progress towards the tasks is outlined in detail below.

Task 1: Evaluate the contribution of BRCA1 and BRCA2 exonic deletions and duplications to breast cancer susceptibility.

We have undertaken analyses for genomic exonic deletions and duplications of *BRCA1* and *BRCA2* in 1500 familial breast cancer cases from separate pedigrees in which mutations of these genes have been excluded. We use a simple, cost-effective copy number analysis technique, multiplex ligation-dependent probe amplification (Schouten et al. 2002; Bunyan et al. 2004).

This analysis has resulted in the identification of genomic duplication / deletion abnormalities in ~ 4% breast cancer families.

Our analyses have demonstrated that:

- MLPA is a cheap, high-throughput and robust technique for copy-number variations, in most situations.
- MLPA should be undertaken in addition to sequencing in all breast cancer families.
- Certain probes show inter-assay variability. We have informed the manufacturers of this and the probes have been replaced.
- Single exon deletions must be further investigated and confirmed – firstly by sequencing to exclude a small exonic mutation under the probe, and if this is normal, by another copy-number assay such as quantitative PCR.
- The clinical features and risks of cancer are the same for families with genomic deletions / duplications as for intragenic mutations.

This strategy is being followed in diagnostic services throughout the UK and in many places internationally.

This project is now complete.

Task 2. Perform familial case-control analyses of non-synonymous coding single nucleotide polymorphisms (SNPs) in DNA repair genes in familial breast cancer cases, Months 1-36:

- a) *Complete identification of coding SNPs by full gene screening of ~50 DNA repair genes in 96 non-BRCA1/2 familial breast cancer cases.*
- b) *Analyse all non-synonymous coding SNPs identified in (a) in 500 additional non-BRCA1/2 familial breast cancer cases and 500 controls.*
- c) *Analyse SNPs that show positive association with breast cancer in (b) in 10,000 unselected breast cancer cases and 10,000 controls.*

We have altered the design of our study to take advantage of technical improvements, more competitive pricing and an international consortium of ~ 30,000 cases and 30,000 controls (Breast Cancer Association Consortium, BCAC) that we are part of and that has been set-up to evaluate variants. This has allowed us to combine Tasks 2 and Task 4 (Identification genome-wide familial case-control analyses) as follows:

- We have identified 114 non-synonymous coding single nucleotide polymorphisms (SNPs) in DNA repair genes through our sequencing of DNA repair genes in 96 *BRCA1/2* negative cases. Probes were successfully designed for 92 of these.
- We included these 92 probes in an array that also included 14,389 non-synonymous coding SNPs that were available from the databases.
- We analysed the 14471 SNPs in 864 familial breast cancer cases and 1498 controls. These results have identified a number of interesting candidates that we are now pursuing.
- The top SNPs in the DNA repair genes we are analyzing in our full series of 1500 familial cases and 2000 additional controls. Those SNPs still showing an association after this

second round will be evaluated through the BCAC consortium to confirm the association and provide cancer risks in familial and non-familial cancer cases. We were part of a recent BCAC collaborative study based on a similar design that demonstrated that a non-synonymous SNP in CASP8 is associated with breast cancer (Cox et al. 2007).

- The top 5% of SNPs from the first array we are planning to analyse in a further 1000 familial cases and 1000 controls using a custom array. The top 30 we will analyse as part of BCAC to confirm true associations. A similar designed complementary study using genome-wide tag SNPs rather than non-synonymous SNPs (i.e. targeting common variation rather than potentially functional variants) in which we have participated has been submitted for publication.

Task 3. Characterise the histopathology and immunohistochemistry of familial breast cancer.

Months 12-36:

- a) *Perform detailed pathological review and immunohistochemical analysis of at least 150 non-BRCA1/2 familial breast cancers.*
- b) *Compare pathology and immunohistochemistry of non-BRCA1/2 familial cancers, BRCA1 cancers, BRCA2 cancers and unselected breast cancers.*
- c) *Define pathological / immunohistochemical characteristics of non-BRCA1/2 cancers which may allow stratification into subgroups that facilitate identification of underlying susceptibility alleles.*

- Within the last year we have identified three new breast cancer predisposition genes (see below). We are therefore focusing on obtaining and characterizing tumors from mutation carriers of these new genes.

- We are undertaking detailed pathology, immunohistochemistry and loss of heterozygosity analyses to define the tumor characteristics associated with the *ATM*, *BRIP1* and *PALB2* mutations.

Task 4. Perform genome-wide familial case-control analyses of non-synonymous coding SNPs,

Months 12-48:

- a) *Analyse ~30,000 non-synonymous coding SNPs (at least 1 from every gene) in 400 non-*BRCA1/2* familial cases and 400 controls.*
- a) *Evaluate top 5% (1500 SNPs) in 800 cases and 800 controls.*

We have undertaken the first phase of this task as outlined above under Task 2. We have been able to increase the size of the study at the same cost, greatly improving the power to detect true associations, due to methodological advancements.

Task 5. Identify low penetrance breast cancer susceptibility alleles, Months 36-60:

- a) *Evaluate top 30-50 SNPs identified in Task 4 in 10,000 unselected breast cancer cases and 10,000 controls to identify which are truly associated with breast cancer and to determine the risks and phenotype in families and isolated breast cancer.*
- b) *Evaluate novel breast cancer susceptibility alleles in *BRCA1* / *BRCA2* / *CHEK2** 1100delC families to determine whether they modify or interact with these genes in breast cancer.*

- We have been undertaking an additional approach to identification of low penetrance breast cancer genes: mutational screening of candidate genes in familial case-control analysis. We have been focusing on DNA repair genes that interact with the known breast cancer genes. In 2006 we completed two of these studies which demonstrate that

mutations in *ATM* and *BRIP1* (also known as *FANCJ*) are lower penetrance breast cancer susceptibility alleles, ~doubling the breast cancer (Renwick et al. 2006; Seal et al. 2006 – see attached papers).

- Through analyses of Fanconi anemia (part of my childhood cancer research) we identified that biallelic *PALB2* mutations cause a new subtype of Fanconi anemia FA-N, which is very similar to FA-D1 which is caused by biallelic *BRCA2* mutations (Reid et al 2007, see attached paper). This raised the possibility that monoallelic *PALB2* mutations might be associated with increased risk of breast cancer, which we were able to demonstrate using our familial case-control strategy (Rahman et al. 2007, see attached paper).
- We are now investigating how mutations in these genes interact with *BRCA1* and *BRCA2*

KEY RESEARCH ACCOMPLISHMENTS

- 1) We have identified three new low penetrance breast cancer predisposition genes, *ATM*, *BRIP1* and *PALB2*.
- 2) We have analysed 14,471 non-synonymous coding SNPs in 864 familial BRCA1/2-negative breast cancer cases and 1498 controls.

REPORTABLE OUTCOMES

We have published three papers in *Nature Genetics* reporting our findings in the last year and a review in *Oncogene*.

CONCLUSION

We have had an exceptionally productive year and received an Outstanding rating for both past and future work at our recent Quinquennial review. We have made substantial progress towards our goals and have been able to improve the power of the studies considerably. We are ensuring that our unique sample resources are being used for maximum benefit by participating in International consortia analyses as well as undertaking our own research. We anticipate that rest of the programme will proceed on course and are hopeful of further discoveries.

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APPENDICES

Four publications – attached

ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles

Anthony Renwick¹, Deborah Thompson², Sheila Seal¹,
 Patrick Kelly¹, Tasnim Chagtai¹, Munaza Ahmed¹,
 Bernard North¹, Hiran Jayatilake¹, Rita Barfoot¹,
 Katarina Spanova¹, Lesley McGuffog², D Gareth Evans³,
 Diana Eccles⁴, The Breast Cancer Susceptibility
 Collaboration (UK), Douglas F Easton², Michael R Stratton^{1,5} &
 Nazneen Rahman¹

We screened individuals from 443 familial breast cancer pedigrees and 521 controls for *ATM* sequence variants and identified 12 mutations in affected individuals and two in controls ($P = 0.0047$). The results demonstrate that *ATM* mutations that cause ataxia-telangiectasia in biallelic carriers are breast cancer susceptibility alleles in monoallelic carriers, with an estimated relative risk of 2.37 (95% confidence interval (c.i.) = 1.51–3.78, $P = 0.0003$). There was no evidence that other classes of *ATM* variant confer a risk of breast cancer.

ATM is a protein kinase that has a key role in monitoring and repair of double-strand DNA breaks. Biallelic mutations in *ATM* cause the autosomal recessive disease ataxia-telangiectasia. Over 70% of *ATM* mutations that cause ataxia-telangiectasia are base substitutions, insertions or deletions that generate premature termination codons or splicing abnormalities¹ (see http://www.benaroyaresearch.org/bri_investigators/atm.htm). Studies of individuals with ataxia-telangiectasia have suggested that female relatives heterozygous for an *ATM* mutation have a two- to fivefold increase in risk of breast cancer^{2,3}. A key prediction of this hypothesis is that heterozygosity for *ATM* mutations (that is, heterozygosity for variants in *ATM* that cause ataxia-telangiectasia) is more common among individuals with breast cancer than the general population. However, studies of breast cancer case and control series have

failed to show an elevated frequency of truncating *ATM* mutations in individuals with breast cancer^{4–6}. These results have prompted alternative models of the role of *ATM* in breast cancer susceptibility. It has been proposed that missense variants (in particular, variants that do not cause ataxia-telangiectasia) predispose to breast cancer⁷. It has also been suggested that only a subset of *ATM* mutations, defined by specific biological characteristics, confer a risk of breast cancer, and that this risk is high, similar to that of mutations in *BRCA1* and *BRCA2* (ref. 8). Finally, it has been proposed that the elevated frequency of breast cancer in mothers of individuals with ataxia-telangiectasia is related to factors other than heterozygosity for *ATM* mutations⁹.

To resolve the confusion regarding the role of *ATM* mutations in breast cancer susceptibility, we adopted a case-control strategy. To maximize the power of the study, we incorporated the following design features. First, we screened genomic DNA from all cases and controls for mutations through the 62 coding exons and splice junctions of *ATM* (Supplementary Methods and Supplementary Table 1 online). This allowed direct and unbiased comparison of the mutation frequency and spectrum in cases and controls. Second, we included only index cases from families with at least three breast cancers. The use of familial, rather than sporadic, breast cancer cases increases the power substantially, as previously illustrated in studies of

Table 1 *ATM* mutations identified in familial breast cancer cases and controls

Family	Mutation	Effect	Number of cases (n = 443)	Number of controls (n = 521)
1	8264delATAAG (8152del117)	Exon 58 skipped	1	0
2	IVS40-1050A→G (5762ins137)	Premature truncation	1	0
3	IVS44+1G→A (6096del103)	Premature truncation	1	0
4	3802delG	Premature truncation	1	0
5	C3349T	Q1117X	1	0
6	5290delC	Premature truncation	1	0
7	790delT	Premature truncation	1	0
8	C7311A	Y2437X	1	0
9	IVS59+1delGTGA (8269del150)	Exon 59 skipped	1	0
10, 11	T7271G	V2424G	2	0
12	TG8565_8566AA	SV2855_2856RI	1	0
	C802T	Q268X	0	1
	6997insA	Premature truncation	0	1

The mutations identified in families 1, 2, 3, 4, 6, 7, 9, 10, 11 and 12 have previously been reported as causative in ataxia-telangiectasia cases^{3,8,13} (http://www.benaroyaresearch.org/bri_investigators/atm.htm). The effect on the transcript of mutations in families 1, 2, 3 and 9 have previously been investigated by RT-PCR and sequencing and are annotated in parentheses after the mutation. The pedigrees of families 1–12 are shown in Figure 1.

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Received 27 March; accepted 9 June; published online 9 July 2006; doi:10.1038/ng1837

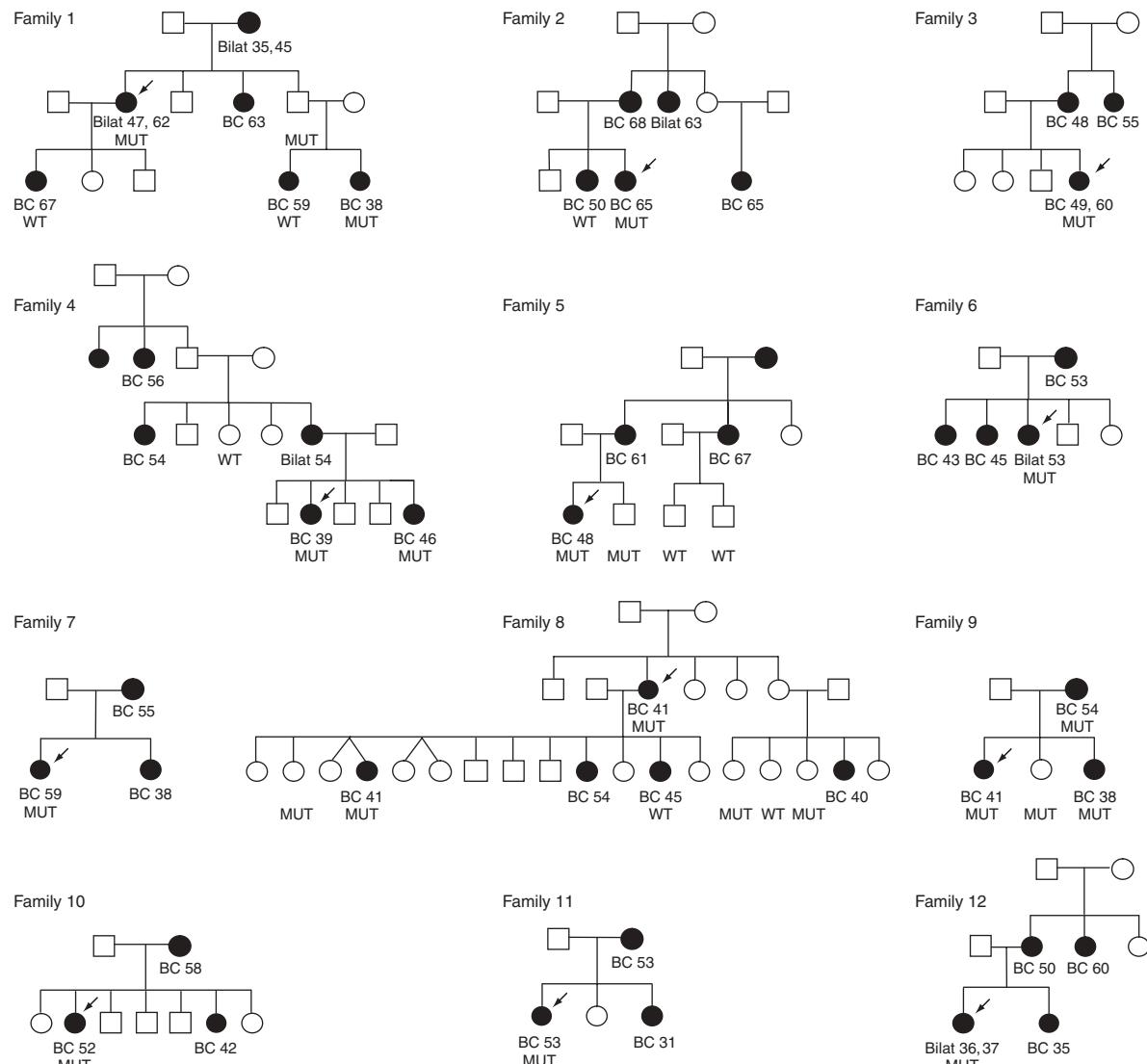


Figure 1 Abridged pedigrees of twelve breast cancer families with *ATM* mutations. Individuals with breast cancer are shown as filled circles, with the age at diagnosis given underneath. If the individual had metachronous bilateral breast cancer, two ages are given. Other cancers or medical conditions are not shown. The index case that was initially screened through *ATM* is shown by an arrow. The *ATM* mutation in each family is given in **Table 1**. BC, breast cancer; Bilat, bilateral breast cancer; MUT, *ATM* mutation present; WT, *ATM* mutation absent.

CHEK2 mutations in breast cancer^{10–12}. Finally, the familial case series had already been pre-screened for *BRCA1* and *BRCA2* mutations and large deletions and duplications. Familial cases due to *BRCA1* or *BRCA2* were excluded, thus enriching the case series for other breast cancer susceptibility alleles (**Supplementary Methods**).

We identified nine (2.04%) *ATM* mutations that result in premature truncation or exon skipping in 443 familial breast cancer cases and two truncating mutations (0.4%) in 521 controls ($P = 0.028$; **Table 1** and **Fig. 1**). All of the mutations are predicted to cause ataxia-telangiectasia, and seven of the nine mutations identified in cases have previously been reported in ataxia-telangiectasia families, including the two most common mutations in the UK, 5762ins137 and 3802delG. The frequency of heterozygotes for truncating *ATM* mutations observed in the control series (0.5%, allowing for a mutation screening sensitivity of 70%) is consistent with that previously estimated for the UK population based on the incidence of ataxia-telangiectasia³.

We also identified 37 different missense variants (**Supplementary Table 2** online). There is strong prior evidence that two of these, V2424G and SV2855_2856RI, are pathogenic mutations in individuals with ataxia-telangiectasia^{3,8,13} (see also http://www.benaroyaresearch.org/bri_investigators/atm.htm and **Supplementary Note** online). Excluding V2424G and SV2855_2856RI, we identified 35 nonsynonymous missense variants, of which 12 were present in both cases and controls, 13 were present exclusively in cases and 10 were present exclusively in controls. None of these has previously been implicated as a disease-causing ataxia-telangiectasia mutation. Five variants (S49C, F858L, P1054R, L1420F, D1853N) had a minor allele frequency of >1% in the combined set; the difference in carrier frequencies between cases and controls was not statistically significant for any of these. Of the remaining 30 rare nonsynonymous missense variants, we found 26 instances in 25 cases, compared with 21 instances in 19 controls ($P = 0.16$). Furthermore, there was no evidence of clustering

of rare nonsynonymous missense variants within conserved ATM functional domains or in the predicted pathogenicity of the variants in cases compared with controls (**Supplementary Note**).

Combining *ATM* truncating, splicing and missense mutations for which there is strong prior evidence of involvement in ataxiatelangiectasia, there were 12 mutations in cases and two in controls ($P = 0.0047$; **Table 1**). The relative risk of breast cancer associated with *ATM* mutations was estimated to be 2.37 (95% c.i. = 1.51–3.78, $P = 0.0003$) by segregation analysis incorporating information from the controls and the full pedigrees of the cases (**Supplementary Methods** and **Supplementary Note**). This estimate is consistent with those derived from studies of ataxiatelangiectasia families and is equivalent to a breast cancer population attributable fraction of 0.86% (95% c.i. = 0.32%–1.72%). There was no evidence of a difference in relative risk between carriers aged below or above 50 years ($P = 0.74$), although the estimated relative risk below age 50 (2.50, 95% c.i. = 1.41–4.17) is consistent with the more substantial risks at young ages suggested by some studies of ataxiatelangiectasia families³. Consistent with the modest estimated relative risk, there was limited evidence of cosegregation of breast cancer with the *ATM* mutation in the five families from which additional samples were available, with five of the nine tested additional individuals with breast cancer carrying the *ATM* mutation present in that family (four expected if the *ATM* mutation were unrelated to breast cancer, $P = 0.36$; **Fig. 1**).

We compared the extent of breast cancer clustering, age at diagnosis and frequency of bilateral breast cancer in index cases with and without *ATM* mutations. The family history of breast cancer was slightly, but not significantly, higher in individuals with *ATM* mutations (median family history score 2.75 versus 2.25, $P = 0.21$). There was no difference in the median age at diagnosis of index cases with an *ATM* mutation (48.6 years) compared with index cases without an *ATM* mutation (48.9 years). The frequency of bilateral cancers was also similar: 1 out of 12 (8%) index cases with an *ATM* mutation developed metachronous bilateral breast cancer, compared with 49/431 (11%) index cases without an *ATM* mutation.

We have previously demonstrated that a truncating mutation in *CHEK2* (*CHEK2*1100delC*) is a breast cancer susceptibility allele conferring a twofold relative risk^{10,11}. We screened the 443 cases and 521 controls in this study for *CHEK2*1100delC* and identified 13 cases and three controls with the mutation ($P = 0.0048$). None of the *ATM* mutation carriers also carried *CHEK2*1100delC*. These data indicate that, in the UK population, the combined *ATM* mutation prevalence is similar to that of *CHEK2*1100delC*; both are associated with similar risks of breast cancer; and both make a similar contribution to breast cancer incidence.

The role of *ATM* in breast cancer susceptibility has been controversial for nearly 20 years. We have now provided strong evidence that *ATM* mutations that cause ataxiatelangiectasia are breast cancer susceptibility alleles. This result is fully consistent with studies of ataxiatelangiectasia families. We did not find evidence of a risk associated with sequence variants not predicted to cause ataxiatelangiectasia. Although we cannot rule out some variation in risk by mutation, the data are consistent with an approximately twofold increase in risk of breast cancer associated with all *ATM* mutations that cause ataxiatelangiectasia.

*Note: Supplementary information is available on the *Nature Genetics* website.*

ACKNOWLEDGMENTS

We thank the participating families who were recruited to the study by the Familial Breast Cancer Collaboration (UK), which includes the following contributors: A. Ardern-Jones, J. Berg, A. Brady, N. Bradshaw, C. Brewer, G. Brice, B. Bullman, J. Campbell, B. Castle, R. Cetnarsryj, C. Chapman, C. Chu, N. Coates, T. Cole, R. Davidson, A. Donaldson, H. Dorkins, F. Douglas, D. Eccles, R. Eeles, F. Elmslie, D.G. Evans, S. Goff, S. Goodman, D. Goudie, J. Gray, L. Greenhalgh, H. Gregory, N. Haites, S.V. Hodgson, T. Homfray, R.S. Houlston, L. Izatt, L. Jeffers, V. Johnson-Roffey, F. Kavalier, C. Kirk, F. Laloo, I. Locke, M. Longmuir, J. Mackay, A. Magee, S. Mansour, Z. Miedzybrodzka, J. Miller, P. Morrison, V. Murday, J. Paterson, M. Porteous, N. Rahman, M. Rogers, S. Rowe, S. Shanley, A. Saggars, G. Scott, L. Side, L. Snadden, M. Steel, M. Thomas, S. Thomas. We thank A. Hall and E. Mackie for coordination of sample collection. We acknowledge use of DNA from the British 1958 Birth Cohort collection, funded by the Medical Research Council grant G0000934 and the Wellcome Trust grant 068545/Z/02. M. Ahmed and B. North are supported by the US Army Medical Research and Material Command grant #W81XWH-05-1-0204. This research was supported by the Institute of Cancer Research and by grants from the Breast Cancer Campaign and Cancer Research UK.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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Truncating mutations in the Fanconi anemia J gene *BRIP1* are low-penetrance breast cancer susceptibility alleles

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We identified constitutional truncating mutations of the *BRCA1*-interacting helicase *BRIP1* in 9/1,212 individuals with breast cancer from *BRCA1*/*BRCA2* mutation-negative families but in only 2/2,081 controls ($P = 0.0030$), and we estimate that *BRIP1* mutations confer a relative risk of breast cancer of 2.0 (95% confidence interval = 1.2–3.2, $P = 0.012$). Biallelic *BRIP1* mutations were recently shown to cause Fanconi anemia complementation group J. Thus, inactivating truncating mutations of *BRIP1*, similar to those in *BRCA2*, cause Fanconi anemia in biallelic carriers and confer susceptibility to breast cancer in monoallelic carriers.

© Breast cancer is approximately twice as common in sisters and mothers of affected individuals as in the general population. Inactivating mutations in *BRCA1*, *BRCA2*, and *TP53* confer a high risk of developing breast cancer (10- to 20-fold by age 60), whereas inactivating mutations of *CHEK2* and *ATM* are associated with more modest risks (approximately twofold). Together, these susceptibility genes are estimated to account for up to 25% of the familial risk of breast cancer. Therefore, most familial aggregation of breast cancer remains unexplained¹.

To identify additional breast cancer susceptibility genes, we screened several genes encoding proteins that interact with the products of known breast cancer predisposition genes. *BRIP1* (also known as *BACH1*) encodes a DEAH helicase that interacts with the BRCT domain of *BRCA1* and has *BRCA1*-dependent DNA repair and checkpoint functions^{2,3}. Inactivating mutations in *BRCA1* predispose to breast cancer. Inactivation of *BRIP1* results in abrogation of certain *BRCA1* functions, and therefore it is plausible that inactivating *BRIP1* mutations also predispose to breast cancer^{4,5}. To investigate this hypothesis, we screened the full coding sequence and intron-exon

boundaries of *BRIP1* by conformation-sensitive gel electrophoresis (CSGE) in genomic DNA from 1,212 women with breast cancer and 2,081 controls (Supplementary Methods and Supplementary Table 1 online). All the individuals with breast cancer had a family history of at least one first-degree relative with breast cancer or equivalent and/or a relative with ovarian cancer. Additionally, all affected individuals were negative for mutations and large deletions or duplications of *BRCA1* and *BRCA2* (see Supplementary Methods for full description of case and control series and mutational analyses of *BRCA1*, *BRCA2* and *BRIP1*). The use of this familial case-control design increases the power substantially¹.

We identified five different truncating mutations in nine of the 1,212 individuals with breast cancer, compared with two truncating mutations in the 2,081 controls ($P = 0.0030$; Table 1 and Fig. 1). There was no evidence of a difference in likelihood of carrying a *BRIP1* mutation between probands with bilateral or unilateral cancers ($P = 0.63$) or by extent of family history of breast cancer ($P = 0.31$). We estimated the relative risk of breast cancer associated with truncating *BRIP1* mutations to be 2.0 (95% confidence interval (c.i.) = 1.2–3.2; $P = 0.012$) by segregation analysis, incorporating information from the controls and the full pedigrees of the affected individuals (Supplementary Methods). The relative risk for carriers aged less than 50 years was 3.5 (95% c.i. = 1.9–5.7), which was significantly higher than the relative risk for carriers above this age ($P = 0.020$). Consistent with the modest estimated relative risk,

Table 1 *BRIP1* mutations identified in individuals with breast cancer and controls

Family	Mutation	Effect	Number of affected individuals ($n = 1,212$)	Number of controls ($n = 2,081$)
1	141delC	Premature truncation	1	0
2–6	2392C → T	R798X	5	1
7	IVS17+2insT	Exon 17 or exon 18 skipped	1	0
8	2008insT	Premature truncation	1	0
9	2255delAA	Premature truncation	1	0
	2108delAinsTCC	Premature truncation	0	1

The mutations identified in families 2–6, 7 and 9 have previously been reported as causative in Fanconi anemia subtype J^{6–10}. The effect on the transcript of the mutation in family 3 has previously been investigated by RT-PCR and sequencing; it results in either deletion of exon 17 or deletion of exon 18 (ref. 8). The pedigrees of families 1–9 are shown in Figure 1.

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Received 10 August; accepted 11 September; published online 8 October 2006; doi:10.1038/ng1902

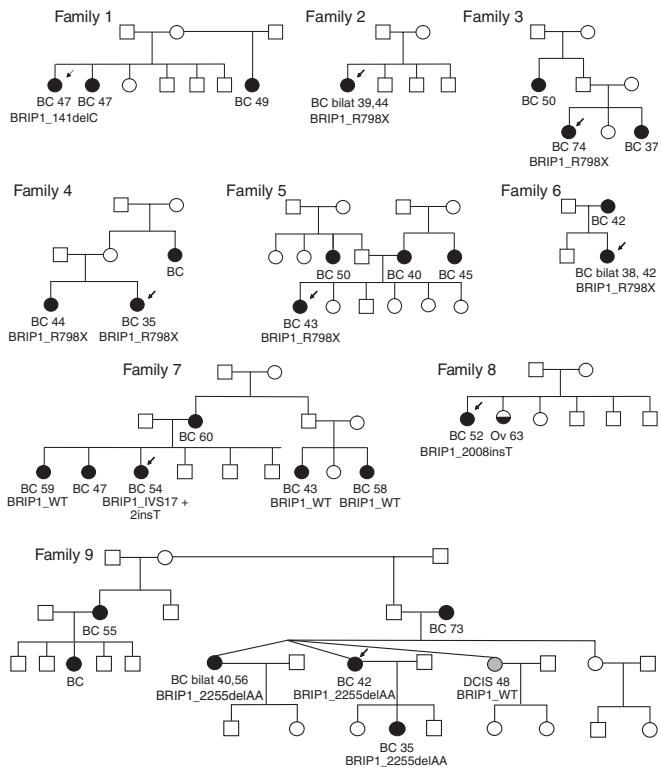


Figure 1 Abridged pedigrees of nine breast cancer families with *BRIP1* mutations. Individuals screened for *BRIP1* mutations are indicated by arrows. Individuals with breast cancer are shown as filled circles, with the age at diagnosis given underneath. An individual with ductal carcinoma *in situ* but no invasive cancer is shown as a shaded circle. If the individual had metachronous bilateral breast cancer, two ages are given. Other cancers or medical conditions are not shown. Samples were not available from individuals with breast cancer that are not genotyped. The *BRIP1* mutation in each family is given in **Table 1** and listed below the individual. BC, breast cancer; BC bilat, bilateral breast cancer; Ov, ovarian cancer; DCIS, ductal carcinoma *in situ*; BRIP1_WT, *BRIP1* mutation absent. We obtained informed consent from all families, and the research was approved by the London Multicentre Research Ethics Committee (MREC/01/2/18).

function *in vitro*, it is unlikely to confer a risk of breast cancer similar to that of truncating mutations.

While we were conducting this study, biallelic inactivating *BRIP1* mutations were reported as the cause of Fanconi anemia complementation group J (FA-J)^{8–10}. Three of the six truncating *BRIP1* mutations we identified were also reported in Fanconi anemia patients. This includes the commonest *BRIP1* mutation in FA-J cases, R798X, which we identified in five separate breast cancer families and one control. None of the FA-J families were reported to have a strong family history of breast cancer consistent with the modest increased risk of breast cancer conferred by *BRIP1* mutations. Moreover, no FA-J case with P47A has been reported, further suggesting that this variant may not be associated with the same cancer risks as truncating mutations. Of note, biallelic mutations of the breast cancer susceptibility gene *BRCA2* have been shown to cause Fanconi anemia complementation group D1 (FA-D1)¹¹.

There are currently 11 known Fanconi anemia genes, and at least one additional gene (underlying complementation group I) awaits identification¹². Epidemiological surveys of relatives of individuals with Fanconi anemia from all complementation groups combined have not provided evidence of an association with breast cancer^{12,13}. However, FA-D1 and FA-J are rare subtypes, and therefore the risks of breast cancer they confer could easily be obscured in studies of all Fanconi subtypes together. Indeed, we have previously analyzed the genes underlying FA-A, FA-C, FA-D2, FA-E, FA-F and FA-G (which together account for over 90% of Fanconi anemia cases) in 88 familial breast cancer cases, and we did not identify any truncating mutations¹⁴. More extensive mutational surveys of FA genes in individuals with breast cancer are now indicated. Notably, however, 8 of the 11 known FA genes encode proteins that form a nuclear core complex that mediates the monoubiquitination of FANCD2. In contrast, *BRCA2* and *BRIP1* are Fanconi anemia genes encoding proteins that function downstream of FANCD2 (ref. 12).

Despite the functional and genetic similarities between *BRCA2* and *BRIP1*, there are some interesting differences in the phenotypes associated with mutations in these genes. Biallelic *BRCA2* mutations confer a high risk of childhood solid and hematological cancers¹⁵, whereas, to date, only one cancer has been reported in an individual with FA-J who has biallelic *BRIP1* mutations^{8–10}. Monoallelic *BRCA2* mutations confer high risks of breast cancer, whereas monoallelic *BRIP1* mutations confer more modest risks, similar to truncating variants of *CHEK2* and *ATM*^{6,7}. The biological explanations for the differences in cancer risk between *BRIP1* and *BRCA2* are currently unclear.

Five other genes implicated in DNA repair are known to confer susceptibility to breast cancer: *TP53*, *BRCA1*, *BRCA2*, *CHEK2* and *ATM*. These genes, together with *BRIP1*, still account

there was limited evidence of linkage of *BRIP1* truncating mutations with breast cancer in the *BRIP1*-positive pedigrees (Fig. 1). This is the typical, and expected, pattern of low-penetrance susceptibility alleles^{6,7}. On the basis of the population frequency and breast cancer risk derived from our study, *BRIP1* mutations have an estimated breast cancer-attributable fraction of 0.20% (95% c.i. = 0.04%–0.44%) in the UK.

It has previously been suggested that certain *BRIP1* missense variants may confer susceptibility to breast cancer^{2,3}. We identified 24 nonsynonymous *BRIP1* missense variants, of which seven were present in both affected individuals and controls, eight were present exclusively in affected individuals and nine were present exclusively in controls (Supplementary Table 2 online). The P919S variant had allele frequencies of 40.3% in affected individuals and 39.3% in controls ($P = 0.43$). The other 23 variants were each observed in <1.5% of the samples, with no significant difference in the frequency of any single variant or in their combined frequency between affected individuals and controls ($P = 0.29$). There was also no significant difference between affected individuals and controls in the *in silico* predicted effect on protein function or the position of missense variants within the gene (Supplementary Methods). These data indicate that the majority of *BRIP1* missense variants are not associated with a risk of breast cancer comparable to that conferred by truncating variants. However, we cannot exclude the possibility that a small number of specific missense alterations confer susceptibility to breast cancer. Notable in this regard is P47A, which was first reported in an individual with early-onset breast cancer and a strong family history of breast and ovarian cancer². This variant alters a highly conserved residue and has been shown to abolish *BRIP1* helicase activity^{2,3}. It was therefore considered likely that the presence of P47A was causally related to the cancer clustering in the family. However, we identified P47A in four affected individuals and four controls ($P = 0.48$), indicating that, despite the deleterious effect on *BRIP1*

only for a minority of the familial aggregation of breast cancer. However, their close functional interactions suggest that other genes involved in DNA repair processes may also be involved in breast cancer susceptibility.

*Note: Supplementary information is available on the *Nature Genetics* website.*

ACKNOWLEDGMENTS

We thank the participating families who were recruited to the study by the Familial Breast Cancer Collaboration (UK), which includes the following contributors: A. Arderm-Jones, J. Berg, A. Brady, N. Bradshaw, C. Brewer, G. Brice, B. Bullman, J. Campbell, B. Castle, R. Cetnarsryj, C. Chapman, C. Chu, N. Coates, T. Cole, R. Davidson, A. Donaldson, H. Dorkins, F. Douglas, D. Eccles, R. Eeles, F. Elmslie, D.G. Evans, S. Goff, S. Goodman, D. Goudie, J. Gray, L. Greenhalgh, H. Gregory, N. Haites, S.V. Hodgson, T. Homfray, R.S. Houlston, L. Izatt, L. Jeffers, V. Johnson-Roffey, F. Kavalier, C. Kirk, F. Lalloo, I. Locke, M. Longmuir, J. Mackay, A. Magee, S. Mansour, Z. Miedzybrodzka, J. Miller, P. Morrison, V. Murday, J. Paterson, M. Porteous, N. Rahman, M. Rogers, S. Rowe, S. Shanley, A. Saggar, G. Scott, L. Side, L. Snadden, M. Steel, M. Thomas and S. Thomas. We thank A. Hall and E. Mackie for coordination of sample collection. We are grateful for the support of the Daniel Faulkner Trust and the Geoffrey Berger Charitable Trust. We acknowledge use of DNA from the British 1958 Birth Cohort collection, funded by the Medical Research Council grant G0000934 and the Wellcome Trust grant 068545/Z/02. P. Kelly is supported by a grant from the Breast Cancer Campaign. M.A., B.N. and A.E. are supported by US Army Medical Research and Material Command grant #W81XWH-05-1-0204. This research was supported by the Institute of Cancer Research and Cancer Research UK.



AUTHOR CONTRIBUTIONS

The study was designed by N.R. and M.R.S. The molecular analyses were performed by S.S., A.R., P.K., R.B., T.C., H.J., M.A. and K.S. under the direction of N.R. The statistical analyses were performed by D.T., A.E., B.N. and L.M. under the direction of D.F.E. The familial collections were initiated by G.E. and D.E. and were collected by the Breast Cancer Susceptibility Collaboration (UK). The manuscript was written by N.R. and M.R.S.

COMPETING INTERESTS STATEMENT

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REVIEW

ATM and breast cancer susceptibility

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ATM was originally identified by positional cloning as the gene that underlies the autosomal recessive condition ataxia-telangiectasia. The encoded protein plays a central role in the complex processes that repair DNA double-strand breaks. Nearly 20 years ago, epidemiological surveys of relatives of ataxia-telangiectasia cases suggested that female relatives were at modestly increased risk of breast cancer. Subsequently, many studies have tried to clarify the role of *ATM* in breast cancer susceptibility, but have produced inconclusive and/or inconsistent results. Recently, large epidemiological and molecular studies have finally provided conclusive evidence that *ATM* mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles.

Oncogene (2006) **25**, 5906–5911. doi:10.1038/sj.onc.1209873

Keywords: ATM; breast cancer susceptibility; ataxia-telangiectasia; breast cancer genes

Ataxia-telangiectasia

Ataxia-telangiectasia (MIM 208900) is an autosomal recessive condition with an estimated frequency of 1 in 40 000 to 1 in 300 000 in Caucasian populations (Swift *et al.*, 1986). It is characterized by progressive cerebellar ataxia, oculomotor apraxia, frequent infections, choreoathetosis, telangiectasias of the conjunctivae, immunodeficiency, sensitivity to ionizing radiation and an increased risk of malignancy (Chun and Gatti, 2004; Taylor and Byrd, 2005). Individuals with ataxia-telangiectasia are estimated to have a 100-fold increased risk of cancer compared with the general population. Lymphoid cancers predominate in childhood, and epithelial cancers, including breast cancer, are seen in adults (Morrell *et al.*, 1986).

The gene for ataxia-telangiectasia was mapped to chromosome 11q by genetic linkage analysis in 1988 and was identified by positional cloning in 1995 (Gatti *et al.*, 1988; Savitsky *et al.*, 1995). The gene was called *ATM* (ataxia-telangiectasia mutated). Although the condition was initially thought to be genetically heterogeneous and had been delineated into four complementation groups,

ATM mutation analysis revealed that all groups were due to mutations in the same gene (Savitsky *et al.*, 1995).

The majority of ataxia-telangiectasia patients are compound heterozygotes or homozygotes for identifiable *ATM* mutations that have been inherited from each parent. The prevalence of such *ATM* mutations has been shown to be 0.5–1% in Western populations (Swift *et al.*, 1986; Renwick *et al.*, 2006). Over 300 distinct mutations have been reported (see www.benaroyaresearch.org) of which >80% are base substitutions or insertions/deletions that generate premature termination codons or splicing abnormalities. The truncated species is usually unstable and results in absent or severely reduced protein expression. Certain missense mutations can also cause ataxia-telangiectasia, but only account for approximately 10% of *ATM* mutations identified in ataxia-telangiectasia patients. Base substitutions in *ATM* are common and it can, therefore, be difficult to deduce whether a specific missense mutation is causative of ataxia-telangiectasia, particularly if protein expression is not abnormal.

ATM structure and function

The *ATM* gene is located at 11q22.3 and consists of 66 exons, 62 of which encode a protein of 3056 amino acids (Savitsky *et al.*, 1995). *ATM* belongs to a protein family known as the PI3K-related protein kinases (PIKK) (Abraham, 2004). These proteins are characterized by a domain similar to that in phosphatidylinositol 3-kinase and most PIKKs, including *ATM*, are active serine/threonine kinases. *ATM* also contains a C-terminal FAT domain (FRAP, *ATM*, TRAPP), with a highly conserved 35 residue tail known as the FATC domain (Bosotti *et al.*, 2000). This domain appears to be important for regulating the kinase activity of *ATM* and for binding regulatory proteins (Jiang *et al.*, 2006). The N-terminus of *ATM* includes several HEAT domains that may influence interactions with other proteins (Perry and Kleckner, 2003) and a region essential for substrate binding (Fernandes *et al.*, 2005). Other putative motifs, including an incomplete leucine zipper and a proline-rich region that binds c-Abl, have been reported, but are less well characterized (Lavin *et al.*, 2004).

ATM has multiple complex functions including a central role in the repair of DNA double-strand breaks. The response to DNA damage includes numerous

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processes including recognition of damaged DNA, recruitment of repair proteins, signalling to cell cycle checkpoints, transcriptional regulation and activation of apoptosis. ATM is involved in many of these processes. In normal cells ATM exists as inert dimers or multimers. In response to double-strand DNA breaks ATM dissociates to highly active monomers (Bakkenist and Kastan, 2003). During this process, ATM undergoes autophosphorylation on Ser1981 and is recruited to sites of DNA damage where it initiates a signalling cascade through phosphorylation of multiple DNA damage response and cell-cycle proteins, including proteins encoded by breast cancer susceptibility genes such as *TP53*, *BRCA1* and *CHEK2*. Full exposition of the functions of ATM is beyond the scope of this article and the reader is referred to recent reviews (Kurz and Lees-Miller, 2004; Jeggo and Lobrich, 2006; Shiloh, 2006).

Epidemiological evidence for breast cancer risk in *ATM* heterozygotes

Swift first proposed that relatives of ataxia-telangiectasia might be at increased risk of breast cancer nearly twenty years ago (Swift *et al.*, 1987). His analysis of cancer incidence in 110 ataxia-telangiectasia families suggested that the relative risk of cancer was 2.3 for men and 3.1 for women, with breast cancer being the most strongly associated cancer. This observation was clearly of importance to ataxia-telangiectasia families, but also had potential wider significance given that it was estimated that up to 1% of the population might be carriers of an ataxia-telangiectasia predisposing mutation. Thus, even a relatively modest increase in breast cancer risk in carriers could equate to an appreciable population attributable risk.

Subsequent to Swift's initial report, several epidemiological surveys of cancer incidence in relatives of ataxia-telangiectasia cases were conducted and confirmed the increased risk of breast cancer. A review of four of these studies estimated the breast cancer relative risk to be 3.9 (Easton, 1994). Recently, a large study of 1160 relatives of 169 ataxia-telangiectasia patients was published (Thompson *et al.*, 2005b). This comprehensive analysis estimated the overall relative risk of breast cancer in carriers to be 2.23 (95% CI = 1.16–4.28) compared to the general population and was higher in women under 50 years of age at 4.9 (95% CI = 1.90–12.9).

Molecular evidence for breast cancer risk in *ATM* heterozygotes

A key prediction of the observation that relatives of ataxia-telangiectasia cases are at increased risk of breast cancer is that *ATM* mutations should occur at increased frequency in breast cancer cases compared with controls. However, the first analysis of 401 breast cancer cases and 201 controls for *ATM* mutations did not support this (FitzGerald *et al.*, 1997).

A variety of reasons were suggested to explain the apparent contradiction between the epidemiological studies and this initial case-control comparison of *ATM* in breast cancer. The model put forward by Gatti *et al.* (1999) has been most widely embraced and proposed that the *ATM* mutations that confer susceptibility to breast cancer differ from those that occur in ataxia-telangiectasia. Specifically, they proposed that missense *ATM* variants might differ from truncating *ATM* mutations in their effect on ATM activity and hence cancer susceptibility. They predicted that carriers of *ATM* truncations would have 50% of wild-type ATM activity and a normal phenotype. Individuals with two truncations would have essentially no ATM protein and would have ataxia-telangiectasia. By contrast, they postulated that certain *ATM* missense variants might encode functionally abnormal proteins that could act in a dominant-negative fashion, which would therefore have more substantial effects on ATM function than a single truncating mutation. This could result in distinct phenotypic consequences in carriers of *ATM* missense variants, such as cancer susceptibility. However, individuals with two missense variants would not have sufficient abrogation of ATM activity to result in ataxia-telangiectasia (Gatti *et al.*, 1999). Although this model can explain the apparent absence of enrichment of *ATM* truncating mutations in individuals with breast cancer, it does not easily explain the increased risk of breast cancer in female relatives of ataxia-telangiectasia cases.

Over the last decade numerous studies have been performed to try to clarify the role of *ATM* in breast cancer susceptibility (Table 1). The majority of these studies have been inconclusive primarily due to two constraining factors. First, most have included small numbers of cases, partly because *ATM* is large and arduous to screen. Second, very few studies have screened the whole *ATM* gene in both cases and controls, thereby limiting the ability to directly compare the frequency and type of identified sequence variants. This is particularly important with respect to missense variants, which are common throughout the genome. For variants of appreciable prevalence, a comparison of the frequency of the specific variant in cases and controls can be undertaken. However, for rare missense variants, the absence of the specific variant in controls confirms that it is rare but gives very little information about the potential role of the variant in cancer susceptibility. Demonstration of an effect on ATM function is supportive that the variant might be associated with a given phenotype, but does not in itself prove that the variant confers susceptibility to breast cancer.

To clarify the role of *ATM* in breast cancer susceptibility we recently conducted an analysis that attempted to overcome these difficulties (Renwick *et al.*, 2006). We adopted a familial case vs control design using 443 *BRCA1/2* negative familial breast cancer cases and 521 controls. This design gives an approximately fourfold increase in power over unselected breast cancer cases. We also screened the full gene in all cases and all

Table 1 Studies investigating the frequency of *ATM* variants in breast cancer cases

Reference	Number of cases and controls screened
Vorechovsky <i>et al.</i> (1996)	ATM screened in 88 breast cancer cases with a family history of breast cancer or other cancers associated with ataxia-telangiectasia. 45 controls screened for variants found in cases
Shayeghi <i>et al.</i> (1998)	ATM screened in 41 breast cancer cases that developed tissue reactions post-radiotherapy and in 39 controls
Bay <i>et al.</i> (1998)	ATM screened in 18 breast cancer cases from families with breast and gastric cancer
Chen <i>et al.</i> (1998)	ATM screened in 100 familial breast cancer cases
Izatt <i>et al.</i> (1999)	ATM screened in 100 breast cancer cases <40 years. 106 controls screened for variants found in cases
Drumea <i>et al.</i> (2000)	ATM screened in 37 breast cancer cases
Broeks <i>et al.</i> (2000)	ATM screened in 82 breast cancer cases <45 years. 268 controls screened for IVS10-6T>G
Shafman <i>et al.</i> (2000)	ATM screened in 57 bilateral breast cancer cases
Laake <i>et al.</i> (2000)	483 breast cancer cases screened for 6 ATM variants
Teraoka <i>et al.</i> (2001)	ATM screened in 177 breast cancer cases and 81 controls
Dork <i>et al.</i> (2001)	ATM screened in 192 breast cancer cases. 1000 controls screened for variants found in cases
Iannuzzi <i>et al.</i> (2002)	ATM screened in 46 breast cancer cases
Sommer <i>et al.</i> (2002)	ATM screened in 43 breast cancer cases and 43 controls
Maillet <i>et al.</i> (2002)	ATM screened in 94 breast cancer cases diagnosed <40 years. 140 controls screened for variants found in case
Offit <i>et al.</i> (2002)	ATM screened in 37 cases with Hodgkin's disease, 10 of whom also had breast cancer. 128 controls screened for variants found in cases
Allinen <i>et al.</i> (2002)	215 familial breast cancer cases, 85 nonfamilial breast cancer cases and 200 controls screened for eight ATM mutations
Chenevix-Trench <i>et al.</i> (2002)	525 or 262 breast cancer cases and 381 or 68 controls screened for T7271G and IVS10-6T>G, respectively
Spurdle <i>et al.</i> (2002)	1300 breast cancer cases and 600 controls screened for T2119C and C3161G
Bernstein <i>et al.</i> (2003)	1149 breast cancer cases screened for T7271G and IVS10-6T>G
Thorstenson <i>et al.</i> (2003)	ATM screened in approximately 270 individuals with a family history of breast and/or ovarian cancer, 60% of whom had breast cancer and 52 controls. Additional controls screened for L1420F and/or IVS10-6T>G
Sommer <i>et al.</i> (2003)	ATM screened in 47 breast cancer cases and 47 controls
Angele <i>et al.</i> (2003)	ATM screened in 51 breast cancer cases. 203 breast cancer cases screened for 16 specific variants
Bretsky <i>et al.</i> (2003)	428 breast cancer cases and 428 controls screened for 20 variants
Szabo <i>et al.</i> (2004)	961 familial breast cancer cases screened for T7271G and IVS10-6T>G
Lindeman <i>et al.</i> (2004)	496 breast and/or ovarian cancer cases screened for IVS10-6T>G
Buchholz <i>et al.</i> (2004)	ATM screened in 67 breast cancer cases. 940 controls screened for variants found in cases
Tamimi <i>et al.</i> (2004)	1309 breast cancer cases and 1761 controls screened for five ATM haplotypes
Thompson <i>et al.</i> (2005a)	378 or 373 familial breast cancer cases and 775 or 84 controls screened for IVS10-6T>G and L1420F, respectively
Lee <i>et al.</i> (2005)	996 breast cancer cases and 1181 controls screened for five variants
Renwick <i>et al.</i> (2006)	ATM screened in 443 familial breast cancer cases and 521 controls

controls so that the frequency and spectrum of mutations could be evaluated in a direct, unbiased analysis.

We identified two *ATM* mutations that cause ataxia-telangiectasia in controls and 12 in familial breast cancer cases ($P=0.0043$). These mutations included truncations, splice-site abnormalities and two missense mutations that were known to affect protein function and to cause ataxia-telangiectasia (Renwick *et al.*, 2006). These results are fully consistent with epidemiological studies that predict that *ATM* mutations that cause ataxia-telangiectasia should occur at increased frequency in breast cancer.

As both cases and controls were fully screened through the gene, a direct comparison of the frequency and type of missense mutations in cases and controls could be undertaken. We identified 37 nonsynonymous missense variants of which 12 were present in both cases and controls, 15 were present exclusively in cases and 10 were present exclusively in controls. For commoner missense variants such as S49C, F858L, P1054R, L1420F and D1853N there was no difference in variant frequency in cases and controls, consistent with most of the previous studies that have examined these variants (Table 1). Of the rare missense mutations, two were previously known to cause ataxia-telangiectasia, T7271G (V2424G) and SV2855_2856RI, and these were only present in cases. Of note, there was no significant

difference in the frequency of nonsynonymous missense variants that occurred either exclusively in cases or exclusively in controls. Neither was there any significant difference in the predicted pathogenicity or position with respect to functional domains of these variants in cases and controls. These data highlight the difficulty in deducing the phenotypic consequences of sequence changes that do not result in protein truncation. Undoubtedly certain nonsynonymous missense variants can cause ataxia-telangiectasia and it is anticipated that such variants also confer susceptibility to breast cancer. The effects of other missense variants, not already known to be associated with ataxia-telangiectasia, are more difficult to predict, but our data suggest that the majority are not associated with increased risks of breast cancer (Renwick *et al.*, 2006).

The relative risk of breast cancer associated with *ATM* mutations was estimated to be 2.37 (95% CI = 1.51–3.78, $P=0.0003$) by segregation analysis incorporating information from the controls and the full pedigrees of the cases. This is very similar to the risks estimated from epidemiological analyses (Thompson *et al.*, 2005b). Therefore, the recent large epidemiological and molecular analyses of *ATM* have provided entirely consistent data that indicate that *ATM* mutations that cause ataxia-telangiectasia are low penetrance breast cancer susceptibility alleles conferring ~2-fold

risks of breast cancer (Thompson *et al.*, 2005b; Renwick *et al.*, 2006). Moreover, the analyses demonstrate that, in the UK population, the combined *ATM* mutation prevalence is similar to that of *CHEK2*1100delC*, and that *ATM* mutations are associated with similar risks of breast cancer and make a similar contribution to breast cancer incidence as *CHEK2*1100delC* (Renwick *et al.*, 2006; Nevanlinna and Bartek, 2006, this issue).

Future challenges

Although substantial progress has been made in our understanding of the role of *ATM* in breast cancer susceptibility there are several questions that need further investigation, such as the identification of factors that determine breast cancer occurrence in *ATM* mutation carriers, risks of other cancers, whether *ATM* heterozygotes are more sensitive to radiation and the clinical utility/implementation of *ATM* mutation testing.

Age and mutation specific risks

Overall, all *ATM* mutations together confer a ~2-fold risk of breast cancer (Thompson *et al.*, 2005b; Renwick *et al.*, 2006). However, there are indications that factors such as age and the specific mutation may influence breast cancer risk. Epidemiological analyses suggest that the risk of breast cancer in *ATM* carriers is higher in women under 50 years and may be higher in first-degree relatives of ataxia-telangiectasia cases compared to more distantly related *ATM* heterozygotes (Olson *et al.*, 2005; Thompson *et al.*, 2005b). The breast cancer risk in individuals above and below 50 years was not significantly different in the study by Renwick *et al.* (2006) but there was limited power to investigate the age-specific risks of *ATM* mutations. Nevertheless, the estimate was consistent with the higher risks in younger individuals reported in epidemiological studies. The age-specific risks associated with *ATM* mutations will need further investigation.

Another question that needs further research is whether there are mutation-specific differences in cancer risk. One particular mutation, T7271G, has been proposed to have a higher risk of breast cancer (Stankovic *et al.*, 1998). This mutation was originally identified in two families with a mild form of ataxia-telangiectasia associated with slowly progressing ataxia, minimal telangiectasia and fertility and is associated with low levels of kinase activity (Stankovic *et al.*, 1998; Stewart *et al.*, 2001). In the first of the families, two individuals homozygous for T7271G developed breast cancer at 44 and 50 years and their mother, an obligate carrier of the mutation, developed breast cancer at 82 years. In the second family, two brothers with ataxia-telangiectasia were compound heterozygotes for T7271G and a truncating mutation. Three paternal aunts of the brothers developed breast cancer in their 50's one of whom was confirmed to be a heterozygous T7271G carrier (Stankovic *et al.*, 1998). Subsequently,

an Australian family was reported in which five women with breast cancer were heterozygous T7271G carriers. Expression and activity analyses of *ATM* in cell lines from the carriers suggested that the mutation acts in a dominant negative fashion (Chenevix-Trench *et al.*, 2002). It is noteworthy that in our study of *ATM* in familial breast cancer, the only mutation that was identified more than once was T7271G, which was identified in the index case of two families that each included three first-degree relatives with breast cancer. This mutation was not detected in the 528 controls and has been shown to be very rare in several other studies (Chenevix-Trench *et al.*, 2002; Bernstein *et al.*, 2003; Szabo *et al.*, 2004; Renwick *et al.*, 2006). These data suggest that the T7271G mutation may be associated with higher risks of breast cancer, but further studies are required to evaluate this.

Other phenotypic features in *ATM* heterozygotes

The original report of cancer in relatives of ataxia-telangiectasia cases suggested that cancers other than breast cancer, such as stomach, pancreas, bladder and ovarian cancer may also occur at increased frequency in *ATM* heterozygotes (Swift *et al.*, 1976; Swift *et al.*, 1991). Subsequent studies have provided inconclusive and/or inconsistent data about whether/which additional cancers are associated with *ATM* heterozygosity (Geoffroy-Perez *et al.*, 2001; Olsen *et al.*, 2001; Thompson *et al.*, 2005b). Further, larger studies will be required to investigate the role of *ATM* in susceptibility to cancers other than breast cancer.

Whether *ATM* heterozygosity is associated with increased sensitivity to radiation is also unclear and has been the subject of several small studies. Radiosensitivity is a hallmark of ataxia-telangiectasia cells and cells from *ATM* heterozygotes show intermediate sensitivity to ionizing radiation in various assays based on cell survival (Speit *et al.*, 2000; Neubauer *et al.*, 2002). However, it remains unclear whether cells from *ATM* heterozygotes exhibit clinically relevant radiosensitivity *in vivo*. It has also been hypothesized that *ATM* heterozygotes may be over-represented in the proportion of breast cancer cases that exhibit exaggerated acute or late reaction of normal tissues following radiotherapy (Angele *et al.*, 2003; Gutierrez-Enriquez *et al.*, 2004; Meyer *et al.*, 2004). Given the increased risk of breast cancer in *ATM* heterozygotes the response to radiation therapy is of potential clinical importance and requires further investigation.

Clinical implementation

The demonstration that *ATM* mutations predispose to breast cancer could be used in risk stratification in women without cancer, to allow them to make medical and lifestyle choices to reduce their risk of breast cancer. The utility of genetic testing of breast cancer genes in reducing cancer incidence has been comprehensively demonstrated for the *BRCA1* and *BRCA2* genes (Domchek and Weber, 2006, this issue). However, mutations in *BRCA1* and *BRCA2* confer ~15-fold risks

of breast cancer and >70% of women with mutations in these genes will develop the disease. By contrast, women with *ATM* mutations have a ~2-fold risk of breast cancer and only ~15% of women with such mutations will develop the disease. Currently, it is not known what determines which women with *ATM* mutations will develop cancer and this limits the clinical utility of identifying such mutations. However, if *ATM* mutations act multiplicatively or epistatically with other genetic or nongenetic breast cancer susceptibility factors it may be possible to identify women with combinations of low penetrance factors that together give risks similar to those of *BRCA1/2*. Such women may benefit from surveillance and surgical interventions similar to those that have proved so successful in *BRCA1/2* carriers (Domchek and Weber, 2006, this issue). *ATM* mutation status may also be of relevance to the treatment of breast cancer if it influences response to radiation or the efficacy of specific chemotherapies as has been suggested for *BRCA*-deficient tumours (Gudmundsdottir and Ashworth, 2006, this issue).

Conclusions

After nearly 20 years of uncertainty, recent epidemiological and molecular studies have clarified the role of *ATM* in breast cancer and have shown that mutations that cause ataxia-telangiectasia in biallelic carriers (homozygotes) confer susceptibility to breast cancer in monoallelic carriers (heterozygotes). *ATM* is the fifth DNA repair gene, together with *BRCA1*, *BRCA2*, *TP53* and *CHEK2* shown to be involved in breast cancer predisposition. Whereas *BRCA1*, *BRCA2* and *TP53* are associated with high risks of breast cancer, *ATM* and *CHEK2* are associated with more modest risks (Antoniou and Easton, 2006, this issue). The reasons for this are not known and neither is it known how *ATM* mutations interact with other genetic or nongenetic risk factors. Future research that explicates which individuals with *ATM* mutations develop cancer will be crucial to the clinical translation of the now incontrovertible evidence that *ATM* mutations predispose to breast cancer.

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PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene

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PALB2 interacts with BRCA2, and biallelic mutations in PALB2 (also known as *FANCN*), similar to biallelic *BRCA2* mutations, cause Fanconi anemia. We identified monoallelic truncating *PALB2* mutations in 10/923 individuals with familial breast cancer compared with 0/1,084 controls ($P = 0.0004$) and show that such mutations confer a 2.3-fold higher risk of breast cancer (95% confidence interval (c.i.) = 1.4–3.9, $P = 0.0025$). The results show that *PALB2* is a breast cancer susceptibility gene and further demonstrate the close relationship of the Fanconi anemia–DNA repair pathway and breast cancer predisposition.

PALB2 (for ‘partner and localizer of *BRCA2*’) encodes a recently discovered protein that interacts with *BRCA2*, is implicated in its nuclear localization and stability and is required for some functions of *BRCA2* in homologous recombination and double-strand break repair¹. In a paper in this issue, we show that biallelic *PALB2* mutations are responsible for a subset of Fanconi anemia cases characterized by a phenotype similar to that caused by biallelic *BRCA2* mutations². Prompted by these observations, we investigated whether monoallelic *PALB2* mutations confer susceptibility to breast cancer by sequencing the gene in individuals with breast cancer from familial breast cancer pedigrees that were negative for mutations in *BRCA1* and *BRCA2* and controls (Supplementary Methods online).

We identified truncating *PALB2* mutations in 10/923 (1.1%) independently ascertained individuals with familial breast cancer from separate families compared with 0/1,084 (0%) controls ($P = 0.0004$) (Table 1 and Fig. 1a). Nine of the *PALB2* mutations were in the 908 families with female breast cancer only (1.0%). One occurred in the 15 families (6.7%) with cases of both female and male breast cancer ($P = 0.15$). Although this observation requires further investigation, it suggests that *PALB2* mutations may confer a higher relative risk of male breast cancer than female breast cancer, and

BRCA2 mutations are known to confer a high relative risk of male breast cancer³. One proband with a *PALB2* mutation developed melanoma at 47 years of age in addition to breast cancer at 56 years. Apart from this individual, there were no other malignancies other than breast cancer in individuals with *PALB2* mutations. Two of four first-degree affected relatives of probands with *PALB2* mutations also carried a *PALB2* mutation. This pattern of incomplete segregation in affected relatives is typical of susceptibility alleles that confer modestly increased risks and is similar to that reported in breast cancer families carrying *CHEK2*, *ATM* or *BRIP1* mutations^{4–6}.

Segregation analysis incorporating the information from controls and the full pedigrees of the affected individuals estimated the relative risk of *PALB2* mutations to be 2.3 (c.i. = 1.4–3.9, $P = 0.0025$). The relative risk for women under 50 years was 3.0 (95% c.i. = 1.4–5.5), and for women over 50 years it was 1.9 (95% c.i. = 0.8–3.7, $P = 0.35$ for difference in relative risk between the age groups). The median age at diagnosis of individuals with *PALB2* mutations was 46 years (interquartile range (IQR) = 40–51) compared with a median age at diagnosis of 49 years (IQR = 42–55) in individuals with breast cancer without *PALB2* mutations ($P = 0.24$ for difference). These data suggest that the risks of breast cancer associated with *PALB2* mutations may be age dependent, but additional studies will be required to address this question. There was no difference in the extent

Table 1 Cancer history and *PALB2* mutations identified through analyses of individuals with familial breast cancer and controls

Family	Cancer history and age of proband	Number of relatives with breast cancer	<i>PALB2</i> mutation	<i>PALB2</i> alteration
1	Breast cancer, 32 years	2	2386G→T	G796X
2	Breast cancer, 51 years	2 female, 1 male	2982insT	A995fs
3	Breast cancer, 43 years	3	3113G→A	W1038X
4	Breast cancer, 49 years	4	3113G→A	W1038X
5	Breast cancer, 28 years	2	3116delA	N1039fs
6	Breast cancer, 50 years	2	3116delA	N1039fs
7	Breast cancer, 55 years	3	3116delA	N1039fs
8	Breast cancer, 42 years	3	3549C→G	Y1183X
9	Breast cancer, 56 years	3	3549C→G	Y1183X
	Melanoma, 47 years			
10	Breast cancer, 40 years	3	3549C→G	Y1183X

The mutations identified in families 5–10 have previously been reported as causative in individuals with Fanconi anemia subtype N (ref. 2; none of the FA-N families are part of this study). The probands with identical mutations were from separately ascertained families that are not known to be related and are from different parts of the UK. The pedigrees of families 1–10 are shown in Figure 1. We did not find any truncating mutations in sequencing the full *PALB2* coding sequence from 1,084 controls.

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Received 24 October; accepted 8 December; published online 31 December 2006; doi:10.1038/ng1959

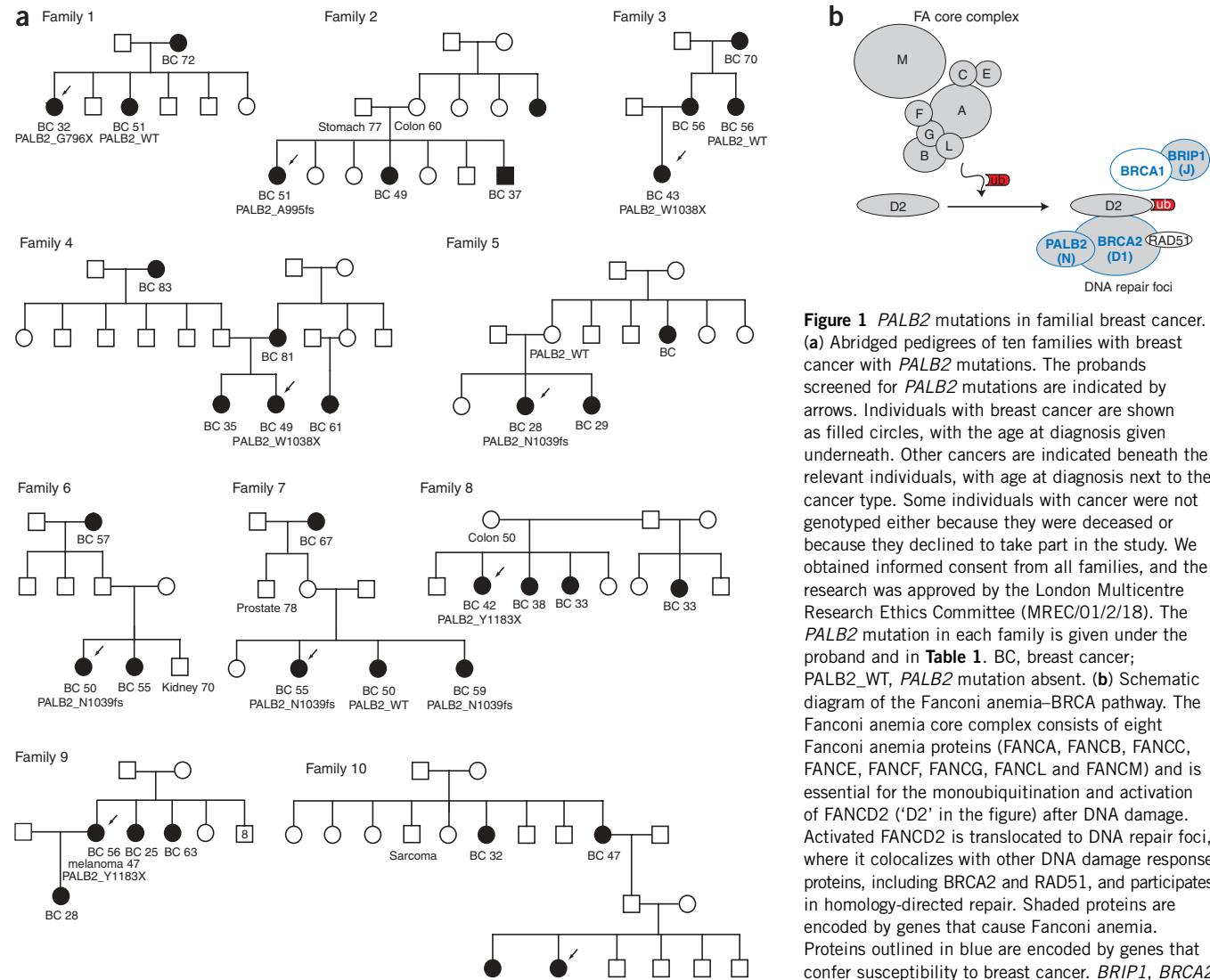


Figure 1 *PALB2* mutations in familial breast cancer. (a) Abridged pedigrees of ten families with breast cancer with *PALB2* mutations. The probands screened for *PALB2* mutations are indicated by arrows. Individuals with breast cancer are shown as filled circles, with the age at diagnosis given underneath. Other cancers are indicated beneath the relevant individuals, with age at diagnosis next to the cancer type. Some individuals with cancer were not genotyped either because they were deceased or because they declined to take part in the study. We obtained informed consent from all families, and the research was approved by the London Multicentre Research Ethics Committee (MREC/01/2/18). The *PALB2* mutation in each family is given under the proband and in Table 1. BC, breast cancer; *PALB2*_WT, *PALB2* mutation absent. (b) Schematic diagram of the Fanconi anemia–BRCA pathway. The Fanconi anemia core complex consists of eight Fanconi anemia proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL and FANCM) and is essential for the monoubiquitination and activation of FANCD2 ('D2' in the figure) after DNA damage. Activated FANCD2 is translocated to DNA repair foci, where it colocalizes with other DNA damage response proteins, including BRCA2 and RAD51, and participates in homology-directed repair. Shaded proteins are encoded by genes that cause Fanconi anemia. Proteins outlined in blue are encoded by genes that confer susceptibility to breast cancer. *BRIP1*, *BRCA2* and *PALB2* are both Fanconi anemia genes and breast cancer susceptibility genes, and they encode proteins functioning downstream of FANCD2.

of familial clustering of breast cancer ($P = 0.69$) or in the probability of being a bilateral case ($P = 0.23$) in families with *PALB2* mutations compared with families without mutations. Assuming a conservative sensitivity of 90% for mutation detection, we estimate the breast cancer population attributable fraction of *PALB2* mutations to be 0.23% (95% c.i.: 0.072%–0.52%) and the percentage of the familial relative risk due to *PALB2* to be 0.24% (0.02%–1.16%).

We identified 50 nontruncating variants within the *PALB2* coding sequence, including 35 nonsynonymous and 15 synonymous variants (Supplementary Table 1 online). There was no overall evidence that *PALB2* missense variants confer susceptibility to breast cancer, with 215 (23%) affected individuals and 265 (24%) controls carrying at least one nonsynonymous missense variant. Only four missense variants had an allele frequency greater than 1%, and there was no evidence that any of these were breast cancer susceptibility alleles. This result is consistent with the data from individuals with Fanconi anemia in which all reported *PALB2* mutations result in premature protein truncation^{2,7}.

Fanconi anemia is a genetically heterogeneous recessive condition that currently includes 13 subtypes, 12 of which have been attributed to distinct genes^{2,8}. The known Fanconi anemia genes encode proteins that interact in an incompletely understood fashion to facilitate recognition and repair of DNA double-strand breaks. A key process in the pathway involves eight of the known Fanconi anemia proteins forming a nuclear core complex that mediates monoubiquitination and activation of FANCD2. Activated FANCD2 is translocated to DNA repair foci, where it colocalizes with BRCA2 and other proteins that effect DNA repair by homologous recombination (Fig. 1b)⁸.

Biallelic mutations of *BRCA2* and *PALB2* cause Fanconi anemia subtypes FA-D1 and FA-N, respectively^{2,7,9}. The phenotypes associated with biallelic *BRCA2* and *PALB2* mutations are markedly similar to each other and differ from the other ten known Fanconi anemia genes. In particular, FA-D1 and FA-N are associated with high risks of solid childhood malignancies such as Wilms tumor and medulloblastoma, which occur very rarely in other subtypes^{2,8,10}. Heterozygous mutations in *BRIP1*, which encodes a *BRCA1*-interacting protein, also

confer an elevated risk of breast cancer⁶, and biallelic *BRIP1* mutations cause Fanconi anemia subtype FA-J^{11,12}. However, FA-J is associated with the classical Fanconi anemia phenotype, and there have not been any reports of individuals with FA-J with a childhood solid tumor^{11,12}.

It is plausible that heterozygosity for mutations in other Fanconi anemia genes may also be involved in breast cancer susceptibility. However, epidemiological studies of relatives of individuals with Fanconi anemia have not demonstrated this, suggesting that breast cancer susceptibility is associated with only a subset of Fanconi anemia genes. This is consistent with the negative results of mutational screens of other Fanconi anemia genes in familial breast cancer cases¹³. The biological features that determine whether a Fanconi anemia gene is also a breast cancer predisposition gene are unknown. However, it is notable that the three Fanconi anemia genes currently associated with breast cancer susceptibility (*BRCA2*, *PALB2* and *BRIP1*) are not part of the Fanconi anemia core complex and are the only known Fanconi anemia genes that act downstream of FANCD2 (Fig. 1b).

We estimate that *PALB2* mutations are associated with an approximately twofold higher risk of female breast cancer. Therefore, despite the fact that *PALB2* is functionally associated with *BRCA2* and that biallelic mutations in both genes cause similar phenotypes, the increase in breast cancer risk associated with *PALB2* monoallelic mutations is clearly more modest than that conferred by *BRCA2* monoallelic mutations, which result in approximately a tenfold increase in risk. These differences in risk are reminiscent of those previously reported between *BRCA1* mutations, which also confer a greater than tenfold increase in risk of breast cancer, and mutations in *BRIP1*, which confer only a twofold increase in risk⁶. The explanations for the apparent differences in risk associated with mutations in these genes, despite the close functional interactions between the proteins they encode, are currently unknown. Thus, our data provide further evidence of the close link between breast cancer susceptibility and the Fanconi anemia-DNA repair pathway, but they also demonstrate that the relationship is complex at both the phenotypic and molecular levels.

With the identification of *PALB2* as a new breast cancer predisposition gene, a clearer picture of the genetic architecture of breast cancer susceptibility is emerging. *BRCA1* and *BRCA2* are likely to be the only major high-penetrance breast cancer susceptibility genes (leading to more than a tenfold higher risk). Mutations in *TP53* also confer high risks of breast cancer but are much rarer¹⁴. These genes are characterized by multiple, rare, inactivating mutations that together account for approximately 15%–20% of the familial risk of the disease¹⁴. A similar mutation spectrum has now been identified in four additional genes that encode proteins that interact biologically with *BRCA1*, *BRCA2* and/or p53. Three of these proteins, *CHK2*, *ATM* and *BRIP1*, interact with *BRCA1*, p53 or both (refs. 8,15). *PALB2* is the first that interacts with *BRCA2*. However, compared with risks associated with mutations in *BRCA1*, *BRCA2* and *TP53*, the risks associated with mutations in *CHEK2*, *ATM*, *BRIP1* and *PALB2* are much lower^{4–6}. Moreover, inactivating mutations in each of these

genes are rare, with fewer than 1% of the population being heterozygotes. As such, the contribution of each gene to the familial risk of breast cancer is small. Collectively, however, they already account for ~2.3% of the overall familial relative risk. Thus, this class of susceptibility gene may make an appreciable contribution to breast cancer predisposition.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

We thank the participating families who were recruited to the study by the Familial Breast Cancer Collaboration (UK) which includes the following contributors: A. Ardern-Jones, J. Berg, A. Brady, N. Bradshaw, C. Brewer, G. Brice, B. Bullman, J. Campbell, B. Castle, R. Cetnarsryj, C. Chapman, C. Chu, N. Coates, T. Cole, R. Davidson, A. Donaldson, H. Dorkins, F. Douglas, D. Eccles, R. Eeles, F. Elmslie, D.G. Evans, S. Goff, S. Goodman, D. Goudie, J. Gray, L. Greenhalgh, H. Gregory, N. Haites, S.V. Hodgson, T. Homfray, R.S. Houlston, L. Izatt, L. Jeffers, V. Johnson-Roffey, F. Kavalier, C. Kirk, F. Laloo, I. Locke, M. Longmuir, J. Mackay, A. Magee, S. Mansour, Z. Miedzybrodzka, J. Miller, P. Morrison, V. Murday, J. Paterson, M. Porteous, N. Rahman, M. Rogers, S. Rowe, S. Sharley, A. Sagar, G. Scott, L. Side, L. Snadden, M. Steel, M. Thomas and S. Thomas. We thank A. Hall and E. Mackie for coordination of sample collection. We acknowledge use of DNA from the British 1958 Birth Cohort collection, funded by the Medical Research Council grant G0000934 and the Wellcome Trust grant 068545/Z/02. P.K. is supported by a grant from the Breast Cancer Campaign. A.E. is supported by US Army Medical Research and Material Command grant W81XWH-05-1-0204. This research was supported by donations from the Geoffrey Berger and Daniel Falkner Charitable Trusts, by the Institute of Cancer Research and by Cancer Research UK.

AUTHOR CONTRIBUTIONS

The study was designed by N.R. and M.R.S. The molecular analyses were performed by S.S., P.K., A.R., S.R., K.S., R.B., T.C., H.J. and S.H. under the direction of N.R. The statistical analyses were performed by D.T., A.E. and L.M. under the direction of D.F.E. The familial collections were initiated by D.G.E. and D.E. and were collected by the Breast Cancer Susceptibility Collaboration (UK). The manuscript was written by N.R. and M.R.S.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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SUPPORTING DATA

None